ab185986 – Human IL-10 SimpleStep ELISA® Kit

For the quantitative measurement of IL-10 in human serum, plasma (citrate), plasma (EDTA), plasma (heparin), and cell culture supernatant.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab185986

This kit is available in a 384-well plate format. This plate utilises smaller volumes of standards and samples per well. Directions for using this format can be found on pg 11.

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity	Storage Condition
Human IL-10 Capture Antibody 10X	600 µL	+4°C
Human IL-10 Detector Antibody 10X	600 µL	+4°C
Human IL-10 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI2	6 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	+4°C
Sample Diluent NBP	20 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 wells	+4°C
Plate Seal	1	+4°C

Note: Antibody Diluent CPI2 – This buffer has been reformulated to enhance stability after freeze-thaw cycles while producing data equivalent to the original formulation of antibody diluent CPI previously used in this kit. While we run stock down, you may receive kits containing antibody diluent CPI. This does not affect the way you should use the kit. If you have any questions, please contact Abcam Scientific Support.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 or 600 nm.
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

Sample Diluent NBP may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

Sample Diluent 50BP: Prepare Sample Diluent 50BP by thoroughly mixing Sample Diluent NBP, then diluting Sample Diluent NBP with 1X Wash Buffer PT. To make 2 mL Sample Diluent 50BP, combine 1 mL 1X Wash Buffer PT and 1 mL Sample Diluent NBP. Mix thoroughly by vortexing and heating gently in a warm water bath until debris is completely dissolved. If required, protease inhibitors can be added.

Sample Diluent 50BP + 1X Enhancer: Prepare Sample Diluent 50BP + Enhancer by thoroughly mixing Sample Diluent NBP, then diluting Sample Diluent NBP and 50X Cell Extraction Enhancer Solution with 1X Wash Buffer PT. To make 10 mL Sample Diluent 50BP + Enhancer, combine 4.8 mL 1X Wash Buffer PT, 5 mL Sample Diluent NBP, and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly by vortexing and heating gently in a warm water bath until debris is completely dissolved. If required, protease inhibitors can be added.

Sample Diluent NS + 1X Enhancer: Prepare Sample Diluent NS + 1X Enhancer by combining Sample Diluent NS and 50X Cell Extraction Enhancer Solution. To make 10 mL Sample Diluent NS + 1X Enhancer, combine 9.8 mL Sample Diluent NS and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CPI2. Mix thoroughly and gently.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. For **serum and plasma samples,** reconstitute the IL-10 standard sample by adding the volume of Sample Diluent 50BP + Enhancer indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 11,550 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 228 µL of Sample Diluent 50BP + Enhancer into tube number 1 and 150 µL of Sample Diluent 50BP + 1X Enhancer into numbers 2-8.

4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	80	228	11,550	3,000
2	Standard#1	150	150	3,000	1,500
3	Standard#2	150	150	1,500	750
4	Standard#3	150	150	750	375
5	Standard#4	150	150	375	187.5
6	Standard#5	150	150	187.5	93.8
7	Standard#6	150	150	93.8	46.9
8	Blank Control	0	150	0	0

1. For **cell culture supernatant samples,** reconstitute the IL-10 standard sample by adding the volume of Sample Diluent NS + 1X Enhancer indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 11,550 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 292 µL of Sample Diluent 50BP + Enhancer into tube number 1 and 150 µL of Sample Diluent NS + 1X Enhancer into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	16	292	11,550	600
2	Standard#1	150	150	600	300
3	Standard#2	150	150	300	150
4	Standard#3	150	150	150	75
5	Standard#4	150	150	75	37.5
6	Standard#5	150	150	37.5	18.8
7	Standard#6	150	150	18.8	9.4
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum*	≤50%
Plasma – Citrate*	≤50%
Plasma – EDTA*	≤25%
Plasma – Heparin*	≤25%
1.5% PHA-Stimulated PBMC Cell Culture Supernatant	3.1 – 50%

*Based on spiked sample

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. To ensure a consistent enhancer concentration, the first dilution must have the appropriate amount of enhancer added in directly from the 50X stock. Dilute samples at least 1:2 into Sample Diluent 50BP **(without enhancer)** add 50X Cell Extraction Enhancer Solution to 1X and assay. Any further dilutions should be made into Sample Diluent 50BP + Enhancer. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. To ensure a consistent enhancer concentration, the first dilution must have the appropriate amount of enhancer added in directly from the 50X stock. Dilute samples at least 1:2 into Sample Diluent 50BP **(without enhancer)** add 50X Cell Extraction Enhancer Solution to 1X and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Any further dilutions should be made into Sample Diluent 50BP + Enhancer. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. To ensure a consistent enhancer concentration, the first dilution must have the appropriate amount of enhancer added in directly from the 50X stock. Dilute samples as needed into Sample Diluent NS **(without enhancer)**, add 50X Cell Extraction Enhancer Solution to 1X and assay. Any further dilutions should be made into Sample Diluent NS + Enhancer. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.

For each assay performed, a minimum of two wells must be used as the zero control.

For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human IL-10.

The standard protein in this kit is mature full length human IL-10.

Native signal was detected in cell culture supernatant.

Spiked protein experiments were used to validate serum, plasma (citrate), plasma (EDTA), and plasma (heparin) sample types.

Saliva, urine, milk, CSF, cell extract, and tissue extract samples have not been tested with this kit.

50% pooled serum and plasma (EDTA, Heparin, Citrate) samples from healthy donors was measured in duplicate. All values were below the detectable range of the assay.

50% serum from ten individual healthy human male donors was measured in duplicate. All values were below the detectable range of the assay.

INTERFERENCE

Recombinant human IL-10 R alpha and human IL-10 R beta were each prepared at 50 ng/mL and 0.6 ng/mL and tested for interference. No interference with was observed.

SPECIES REACTIVITY

Recombinant mouse and rat IL-10 were prepared at 50 ng/mL and 0.6 ng/mL each and assayed for cross reactivity. No cross-reactivity was observed.

Other species reactivity was determined by measuring 50% serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species: Mouse, Rat, Cow

Other species reactivity not determined.

CALIBRATION

This immunoassay is calibrated against a highly purified human IL-10. The NIBSC/WHO unclassified purified human IL-10 preparation 93/722 was evaluated in this kit.

The dose response curve of the unclassified standard IL-10 parallels the SimpleStep standard curve. To convert sample values obtained with the SimpleStep human IL-10 kit to approximate NIBSC 93/722 units, use the equation below.

NIBSC (93/722) approximate value (IU/mL) = 0.01236 x SimpleStep Human IL-10 value (pg/mL).

CALCULATION

- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

  Δ Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at absorbance values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.072	0.069	0.070
46.9	0.124	0.127	0.125
93.8	0.198	0.195	0.196
187.5	0.281	0.297	0.289
375	0.531	0.537	0.534
750	1.003	1.090	1.046
1,500	2.009	1.965	1.987
3,000	3.468	3.442	3.455

Table 1. Example of human IL-10 standard curve in Sample Diluent 50BP + 1X Enhancer. The IL-10 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.085	0.089	0.087
9.4	0.138	0.137	0.138
18.8	0.186	0.189	0.188
37.5	0.271	0.275	0.273
75	0.449	0.458	0.454
150	0.812	0.838	0.825
300	1.528	1.586	1.557
600	2.822	2.783	2.803

Table 2. Example of human IL-10 standard curve in Sample Diluent NS + 1X Enhancer. The IL-10 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent 50BP + 1X Enhancer	25	7.1 pg/mL
Sample Diluent NS + 1X Enhancer	25	1.4 pg/mL

Recovery

Three concentrations of IL-10 were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
50% Serum	109	108 – 112
50% Plasma – Citrate	111	105 – 119
25% Plasma – EDTA	109	103 – 118
25% Plasma – Heparin	116	113 – 117
25% Stimulated PBMC Cell Culture Supernatant	108	105 - 111
95% Cell Culture Media*	106	102 - 111

*Media is RPMI 1640 containing 10% fetal bovine serum.

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Recombinant IL-10 was spiked into the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 50BS + 1X Enhancer.

Dilution Factor	Interpolated value	50% Human Serum	50% Human Plasma (Citrate)	25% Human Plasma (EDTA)	25% Human Plasma (Heparin)
Undiluted	pg/mL	2,410	2,293	2,279	2,110
	% Expected value	100	100	100	100
2	pg/mL	1,078	1,052	1,079	1,042
	% Expected value	89	92	95	99
4	pg/mL	526	527	531	523
	% Expected value	87	92	93	99
8	pg/mL	243	260	251	266
	% Expected value	81	91	88	101
16	pg/mL	118	113	126	123
	% Expected value	83	87	88	93

Native IL-10 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS + 1X Enhancer.

Dilution Factor	Interpolated value	50% Human 1.5% PHA-Stimulated PBMC SN
Undiluted	pg/mL	227
	% Expected value	100
2	pg/mL	119
	% Expected value	105
4	pg/mL	59
	% Expected value	103
8	pg/mL	29
	% Expected value	101
16	pg/mL	14
	% Expected value	101

Precision

Mean coefficient of variations of interpolated values of IL-10 from three concentrations of stimulated PBMC cell culture supernatant within the working range of the assay.

	Intra-assay	Inter-assay
N=	5	3
CV (%)	5.2	3.2

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
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For technical support contact information, visit: www.abcam.com/contactus

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DIRECTIONS FOR 384-WELL PLATE FORMAT:

Materials Supplied for 384-well Format

Item	Quantity	Storage Condition
Human IL-10 Capture Antibody 10X	600 µL	+4°C
Human IL-10 Detector Antibody 10X	600 µL	+4°C
Human IL-10 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI2	6 mL	+4°C
Cell Extraction Enhancer Solution 50X	6 x 1 mL	+4°C
Sample Diluent NBP	20 mL	+4°C
Sample Diluent NS	250 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	2 x 12 mL	+4°C
Stop Solution	2 x 12 mL	+4°C
SimpleStep Pre-Coated 384-Well Microplate	384 wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 or 600 nm in a 384-well plate.
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).
Optional: Automated liquid handler.

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for one full plate. The sample volumes below are sufficient for running all 384 wells; adjust volumes as needed for the number of samples and dilution scheme for your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

Sample Diluent NBP may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

Sample Diluent 50BP: Prepare Sample Diluent 50BP by thoroughly mixing Sample Diluent NBP, then diluting Sample Diluent NBP with 1X Wash Buffer PT. To make 4 mL Sample Diluent 50BP, combine 2 mL 1X Wash Buffer PT and 2 mL Sample Diluent NBP. Mix thoroughly by vortexing and heating gently in a warm water bath until debris is completely dissolved. If required, protease inhibitors can be added.

Sample Diluent 50BP + 1X Enhancer: Prepare Sample Diluent 50BP + Enhancer by thoroughly mixing Sample Diluent NBP, then diluting Sample Diluent NBP and 50X Cell Extraction Enhancer Solution with 1X Wash Buffer PT. To make 30 mL Sample Diluent 50BP + Enhancer, combine 14.4 mL 1X Wash Buffer PT, 15 mL Sample Diluent NBP, and 0.6 mL Cell Extraction Enhancer Solution 50X. Mix thoroughly by vortexing and heating gently in a warm water bath until debris is completely dissolved. If required, protease inhibitors can be added.

Sample Diluent NS + 1X Enhancer: Prepare Sample Diluent NS + 1X Enhancer by combining Sample Diluent NS and 50X Cell Extraction Enhancer Solution. To make 50 mL Sample Diluent NS + 1X Enhancer, combine 49 mL Sample Diluent NS and 1 mL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 100 mL 1X Wash Buffer PT combine 10 mL Wash Buffer PT 10X with 90 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. To make 6 mL of the Antibody Cocktail combine 600 µL 10X Capture Antibody and 600 µL 10X Detector Antibody with 4.8 mL Antibody Diluent CPI2. Mix thoroughly and gently.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. For **serum and plasmas,** reconstitute the IL-10 standard sample by adding the volume of Sample Diluent 50BP + 1X Enhancer indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 11,550 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 114 µL of Sample Diluent 50BP + 1X Enhancer into tube number 1 and 75 µL of Sample Diluent 50BP + 1X Enhancer into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	40	114	11,550	3,000
2	Standard#1	75	75	3,000	1,500
3	Standard#2	75	75	1,500	750
4	Standard#3	75	75	750	375
5	Standard#4	75	75	375	187.5
6	Standard#5	75	75	187.5	93.8
7	Standard#6	75	75	93.8	46.9
8	Blank Control	0	75	0	0

1. For **cell culture supernatants,** Reconstitute the IL-10 standard sample by adding the volume of Sample Diluent NS + 1X Enhancer indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 11,550 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 146 µL of Sample Diluent NS + 1X Enhancer into tube number 1 and 75 µL of Sample Diluent NS + 1X Enhancer into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	8	146	11,550	600
2	Standard#1	75	75	800	300
3	Standard#2	75	75	400	150
4	Standard#3	75	75	200	75
5	Standard#4	75	75	100	37.5
6	Standard#5	75	75	50	18.8
7	Standard#6	75	75	25	9.4
8	Blank Control	0	75	0	0

Plate Preparation

The 384-well plate included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
For each assay performed, a minimum of two wells must be used as the zero control.

For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure for 384-well Plate Format

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Add 12.5 µL of all sample or standard to appropriate wells.
3. Add 12.5 µL of the Antibody Cocktail to each well.
4. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 700 rpm.
5. Wash each well with 3 x 100 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 100 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
6. Add 25 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 700 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
7. Add 25 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading. Proper mixing of the Stop Solution is required for proper measurement.
8. Alternative to 6 – 7: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec – 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 25 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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